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Analysis and characterization of methyl esters of fatty acids of some *Gracilaria* species

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ABSTRACT

The analysis of the total methyl esters of fatty acids from the extracts of *Gracilaria birdiae*, *Gracilaria caudata*, *Gracilaria cerviconis*, *Gracilaria domingensis* collected in the coast of Paraíba, Northeast Brazil, as well as their chemotaxonomic significance are herein reported for the first time. They can be recognized as a group that represents analogous chemotaxonomic basis since they show common constituents in their composition, such as hexadecanoic acid and cholesterol. Differences found in the fatty acid composition between the studied species reinforce the proposal to transfer *G. caudata* for the genus *Hydropuntia* (*H. caudata*).

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1. Subject and source

Marine organisms are potentially prolific sources of highly bioactive secondary metabolites that might represent useful leads in the development of new pharmaceutical agents (Gamal, 2010).

The marine macroalgae are a heterogeneous group of plants found abundantly in all ecosystems (Sousa et al., 2008). Seaweeds are used for great number of application by man mainly as a source of human food and as a source of gums (phycocolloids) (Gamal, 2010).

Red algae are considered as the most important source of many biologically active metabolites in comparison to other algal classes. There are about 8000 species of red algae, most of which are of marine source. These are found in the intertidal and in subtidal to depths of up to 40, or occasionally, 250 m (Gamal, 2010).

Gracilaria and other red algae are used in manufacture of the all-important agar, used widely as a growth medium for microorganisms and biotechnological applications (Gamal, 2010). The gracilarioid algae include some of the most valuable marine plants (Bellorin et al., 2002) and they show significant economic impact (Schmidt et al., 2010). Species of this genus have been extensively studied because of the high utilization of their phycocolloids (Schmidt et al., 2010).

Currently, some authors have disagreed about the taxonomic position of the species *Gracilaria caudata* and *Gracilaria cornea*. Bellorin et al. (2002) recognize these species as valid ones and proposed synonymy between the genera *Gracilaria* and

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Hydropuntia. Liao and Hommersand (2003) mention in a taxonomic review on the family Gracilariaceae that this classification is controversial and is accepted by some and rejected by others. Gurgel and Fredericq (2004) based on morphological and molecular studies validate the genus *Hydropuntia* and transfer several species of *Gracilaria* for the *Hydropuntia* complex, including *G. caudata*.

In northeastern Brazil littoral areas, red seaweeds like *Gracilaria* are abundant (Marinho-Soriano et al., 2007). Four species of *Gracilaria*, *Gracilaria birdiae* Plastino & Oliveira, *Gracilaria cervicornis* (Turner) J. Agardh, *G. caudata* J. Agardh, and *Gracilaria domingensis* (Kuetz.) Sonder ex Dickie were selected for study. Three of the species (*G. birdiae*, *G. caudata*, and *G. domingensis*) were collected from Carapibus beach, municipality of Conde, and one species (*Gracilaria cervicornis*) from Bessa beach, municipality of João Pessoa, State of Paraíba, Northeast Brazil, by Miranda G.E.C. in 2009. Voucher specimens numbers JPB 46007 (*G. birdiae*), JPB 46006 (*G. caudata*), JPB 46008 (*G. cervicornis*) and JPB 46009 (*G. domingensis*) are kept at the Herbarium Lauro Pires Xavier (JPB), Federal University of Paraíba (Cria, 2011).

To the best of our knowledge, this paper reports for the first time the analysis of the total methyl esters of fatty acids from the extracts of *G. birdiae*, *G. caudata*, *G. cervicornis*, *G. domingensis*, and their chemotaxonomic significance.

2. Previous work

There has not been any report on chemotaxonomic classification of *Gracilaria* genus from the coast of Paraíba, Northeast Brazil.

3. Present study

Samples were rinsed with fresh water to eliminate foreign materials such as sand and shells. They were then air-dried to be exhaustively extracted with dichloromethane/methanol (3:1) at room temperature. A sample (500 mg) of each extract was converted to fatty acids methyl esters by hydrolysis. To each extract 10 mL of methanol and 1 mL of hydrochloric acid were added for 1 h at 70 °C and stirred continuously. The resulting extracts were submitted to liquid partition with water and ethyl acetate and the fatty acids in the organic partition were subject to GC–MS analysis.

GC–MS analyses were carried out using a Hewlett–Packard 5973–6890 GC–MS system operating in electron ionisation mode at 70 eV, equipped with a split–splitless injector and a flame ionisation detector. Injection was performed at 200 °C in a split ratio 1:10, while detection was performed at 250 °C. The column employed for the analysis was a HP-5 MS fused silica capillary column (30 m × 0.25 mm; film thickness 0.25 µm). The carrier gas was helium at a flow rate of 2 mL/min. The oven temperature was 60 °C at the time of the injection, raised to 300 °C at a rate of 3 °C/min and subsequently held at 300 °C for 10 min. The relative component concentrations were calculated from total ion chromatographs by a computerized integrator. The identification of the chemical constituents was based on comparison of their retention times and mass spectra with those obtained from authentic samples (Sigma Chemical Co., St. Louis, Mo, USA) and/or reported in the NIST/NBS, Wiley libraries and the literature (Adams, 2001).

Based on the fatty acid composition of the studied species a cluster analysis (Euclidean distance) was performed to identify the similarities between groups. This analysis excluded the occurrence of fatty acids common to all species studied in order to maximize the differences between groups.

Compounds identified in the total fatty acids methyl esters extracts with their relative abundance (%) are listed in Table 1. The hydrocarbon heptadecane was shown to be present in *G. birdiae*, *G. caudata*, *G. cervicornis* and *G. domingensis* in comparable percentages of 1.12%, 0.65%, 1.15%, and 1.15%, respectively. Fig. 1 shows the cluster formed by the analysis of similarity in the composition of their fatty acids. The species *G. cervicornis* and *G. domingensis* formed a group with high similarity, while *G. caudata* was isolated due to its greater difference in composition. *G. birdiae* occupied an intermediate position between these two groupings.

It is important to say that fatty acids are primary metabolites of acetyl CoA pathway which is genetically determined, evolutionary very old, and therefore conservative (Petkov and Garcia, 2007). The major and most representative component in all analyzed species is the methyl ester of the saturated fatty acid hexadecanoic acid (palmitic acid) which is exhibited in high percentage in *G. birdiae* (41.86%), *G. caudata* (21.34%), *G. cervicornis* (50.94%) and *G. domingensis* (13.46%). Strengthening our data, according to the literature, the genus *Gracilaria* has as the highest amount of saturated fatty acid the palmitic acid (Gressler et al., 2010; Khotimchenko, 2005; Norziah and Ching, 2000; Vaskovsky et al., 1996; Wen et al., 2006). In addition, evidence on literature has shown that palmitic acid isolated from a marine red alga may be a lead compound of anticancer drugs (Harada et al., 2002). The methyl esters of tetradecanoic, 9-octadecenoic, and octadecanoic acids were also identified in all extracts.

The analysis on *G. caudata* allowed the classification of the highest quantity of constituents. Among them, it is worth mentioning the presence of cholesterol (Cholest-5-en-3-ol) in an amount of 0.49%. *G. birdiae* was also shown to have this compound in a percentage of 0.26%. This result is consistent with previous studies since cholesterol and other C-27 sterols are considered the primary sterols of the red algae (Fattorusso et al., 1975; Gibbons et al., 1967; Govindan et al., 1993; Hattab et al., 2006; Moses Babu et al., 1990). Besides, cholesterol has also been found in other species of *Gracilaria* (Das and Srinivas, 1992a, 1992b; Govindan et al., 1993; Henriquez et al., 1972; Sims and Pettus, 1976).

Table 1

Chemical composition from four species of *Gracilaria* (*G. birdiae*, *G. caudata*, *G. cervicornis*, *G. domingensis*) based on the analysis of their total methyl esters of fatty acids.

Compound name	RT	Relative abundance			
		<i>G. birdiae</i>	<i>G. caudata</i>	<i>G. cervicornis</i>	<i>G. domingensis</i>
2-Heptenal, trans-2-hepten	6.13	–	0.06	–	–
Dodecanoic acid, methyl ester (C12:0)	29.17	–	0.01	–	–
n-Heptadecane	36.18	1.12	0.65	1.15	1.15
Tetradecanoic acid, methyl ester (C14:0)	37.15	5.64	2.19	5.32	2.18
Pentadecanoic acid, methyl ester (C15:0)	40.74	–	0.32	0.57	–
2-Pentadecanone, 6,10,14-trimethyl	41.30	–	0.22	0.36	–
9-Hexadecenoic acid, methyl ester (C16:1)	43.49	–	0.63	0.76	–
Hexadecanoic acid, methyl ester (C16:0)	44.71	41.86	21.34	50.94	13.46
Heptadecanoic acid, methyl ester (C17:0)	47.75	TR	0.12	–	–
9,12-Octadecadienoic acid, methyl ester (C18:2)	49.77	–	–	–	0.19
8, 11-Octadecadienoic, methyl ester (C18:2)	49.94	0.38	0.29	TR	–
9-Octadecenoic acid, methyl ester (C18:1)	50.18	2.87	0.95	4.66	1.58
13-Octadecenoic acid, methyl ester (C18:1)r	50.34	2.03	3.08	–	–
Octadecanoic acid, methyl ester	50.87	1.40	1.58	1.88	1.46
6,9-Octadecadienoic acid, methyl ester (C18:2)	52.77	0.51	–	TR	–
7,10-Octadecadienoic acid, methyl ester (C18:2)	52.91	–	0.13	TR	–
Cyclopropane octanoic acid, 2-octyl-, methyl ester	53.57	–	1.17	–	–
5,9,11,14-Eicosatetraenoic acid, ethyl ester (C20:4)	54.68	–	–	0.61	1.81
5,8,11,14-Eicosatetraenoic acid, ethyl ester (C20:4)	54.77	4.07	–	–	–
Methyl arachidonate 5,8,11,14-eicosatetraenoic acid	55.13	–	6.13	–	–
7,10,13-Eicosatrienoic acid, methyl ester (C20:3)	55.28	0.37	0.40	–	–
11,13-Eicosadienoic acid, methyl ester (C20:2)	56.01	TR	0.19	–	–
Eicosanoic acid, methyl Ester (C20:0)	56.96	–	0.18	TR	–
Phosphoric acid, triphenyl ester	58.57	TR	0.23	TR	0.23
Tetracosanoic acid, methyl ester (C24:0)	67.63	TR	0.07	–	–
Cholesta-3,5-diene, Cholesterile	71.11	TR	0.13	–	0.50
Cholest-5-en-3-ol (3,beta)	76.26	0.26	0.49	–	–
Cholest-4-ene-3-one	78.92	–	0.05	–	–
Total identified		60.51%	40.61%	66.25%	22.56%

TR, traces.

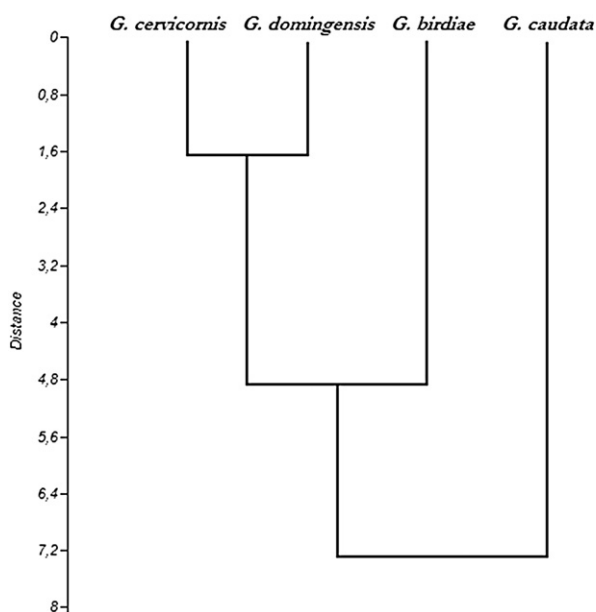


Fig. 1. Cluster analysis of the fatty acid composition between the species studied, *G. cervicornis*, *G. domingensis*, *G. caudata* and *G. birdiae*. (y-axis = Euclidean distance).

4. Chemotaxonomic significance

The chemical composition of the total methyl esters of fatty acids from the extracts of four species of *Gracilaria* showed to have very similar profile. The hydrocarbon heptadecane together with the methyl esters of tetradecanoic, 9-octadecenoic, octadecanoic and hexadecanoic acids were identified in all extracts, being the latter the major compound which is according to literature data.

This study shows that the species *G. birdiae*, *G. caudata*, *G. cervicornis* and *G. domingensis* collected in Paraíba coast, Northeast from Brazil, can be recognized as a group that represents analogous chemotaxonomic basis which is characteristic of the family Gracilariaceae. The results demonstrate the phylogenetic proximity between the species *G. cervicornis* and *G. domingensis* which are considered sister groups by authors such as Bellorin et al. (2002) and Gurgel and Fredericq (2004) based on morphological and molecular studies. Regarding the taxonomic position of *G. caudata*, this study isolated this species from the others thus reinforcing the proposal of Gurgel and Fredericq (2004) that transferred it to the genus *Hydropuntia*. *G. birdiae* described by Plastino and Oliveira (2002) occupied an intermediate position between the groups and as long as it was not studied on the phylogenetic point of view there can be no comparison between the fatty acid composition and the phylogenetic position.

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